European Journal of Biotechnology and Bioscience
ISSN: 2321-9122; Impact Factor: RJIF 5.44
Received: 25-02-2020; Accepted: 20-03-2020; Published: 27-03-2020
www.biosciencejournals.com
Volume 8; Issue 2; 2020; Page No. 01-03

Protective effect of *Nyctanthes arbortristis* leaf extract on the genotoxicity of mice induced by profenofos

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Abstract
The present research was designed to evaluate the possible protective potentials of Nyctanthes arbortristis at two different concentrations against the profenofos induced genotoxicity in Swiss albino mice. Profenofos when administered 2mg/ml orally to Swiss albino mice for 30 days increased the frequency of abnormality compare to control in mitotic chromosome. When mice fed only with *Nyctanthes arbortristis* leaf extract, the abnormality was all most equal to the control group. However, when profenofos with *Nyctanthes arbortristis* leaf extract fed concurrently, the abnormality was minimized comparison to profenofos treated group at both concentrations of *Nyctanthes arbortristis* leaf extract, but higher concentration of leaf extract was significantly minimized the genotoxicity comparison to lower concentration of leaf extract. Therefore, it is suggested that leaf extract may reduce the risk of profenofos induced genotoxicity in mice.

Keywords: *Profenofos, Nyctanthes arbor tristis, Antioxidant, Genotoxicity, Swiss albino mice*

1. Introduction
Owing to the great potentialities of the pesticides in protecting crops right from the seedling stage upto harvesting and even when stored as grains, their use is increasing day-by-day. There is no doubt in usefulness of pesticide in solving problem of feeding the ever-increasing population. There are, however, certain darker aspects of pesticide use which demand our immediate attention. Whenever pesticides are sprayed, the plant parts absorb them from their external surface. The pesticide residues most often persist for sufficiently long time inside the plant body. Considering human as the primary consumer of agricultural produce it becomes very clear that he is being chronically exposed to the hazards of very - very small doses of pesticide residues.
Profenofos is an organophosphate pesticide used for various agricultural and household purpose to control insects and other pests [1, 2]. These profenofos are potentially hazardous to non-targeted animals, humans and other organisms. A number of studied have been conducted to investigate the genotoxic effect [3, 4], mutagenic effect [5] and carcinogenic effect [6] of organophosphate pesticide. Organophosphates have been widely studied for their ability to induced damage to DNA and have demonstrated genotoxic and clastogenic properties [7, 8, 9, 10]. Profenofos known to induce chromosomal aberration [11, 12] in somatic and germ cell and micronuclei and histopathological changes [13] in mice. Therefore, at the moment the prevention of pesticide use may only be achieved if compounds which derived from medicinal plants and their products.
Plants have been used for the treatment of various diseases for thousands of years, all over the world [14]. There are many reports showing the rising trends of anti- mutagenic studies with plant extracts, such as Aloe vera has got limited protective value against arsenic induced oxidative stress [15]. Aqueous extract of Amla have been found to show protective role against cytotoxic effect of lead and aluminium salt [16] and arsenic [17]. A number of plant products as aqueous Neem leaf extract [18], Moringa oleifera [19], Papaya fruit extract [20] and Mentha specieta extract [21] reported to have beneficial effect against genotoxicity. Anti-mutagenic and anti-carcinogenic property of a wide variety of dietary constituent and plant secondary metabolites have also been reported [22, 23] due to presence of antioxidants. *Nyctanthes arbor tristis* is the most popular, commonly used, versatile medicinal plant which commonly known as harsingar. It has antimicrobial, anti-inflammatory, hepatoprotective, anti- puretic, antioxidant, antifungal, anti-parasitic, anti-malarial and anti-diabetic activities [24, 25, 26].
Recent studies has shown that leaves of *Nyctanthes arbor tristis* is a good source of natural antioxidant and they contain flavonoids, tannins, glycosides as a phenolic compounds phenolic, these phenolic compounds are antioxidant agent which shows free radical scavenging activity[27]. Therefore, the present work was under taken to study the protective effect of *Nyctanthes arbor tristis* leaf extract against the profenofos induced genotoxicity through the chromosomal aberration in bone marrow cell of mice.

2. Materials and Methods
Four to five week (25-30g) old Swiss albino mice were obtained from the laboratory inbred stock and maintained in the animal house of the P.G. Department of the Zoology and kept under the standard laboratory condition. The animals were fed on food grains and tap water. Treatment and protocols employed in this study were done after proper approval of the institutional Head and Departmental research community.

2.1 Treatment
The mice were separated into six groups and subjected to treatment for 30 days. First group was treated as a control. Second group was treated with profenofos pesticide (2mg/ml). Third and fourth groups were treated with

Nyctanthes arbor tristis leaf extract (10ml/kgb.w) at two different concentration (25%concentration as a lower dose and 75% concentration as a higher dose). Fifth and sixth groups of mice were treated with *Nyctanthes arbor tristis* leaf extract at both 25% concentration and 75% concentration respectively along with profenofos as a concurrent dose (Table-1). Profenofos was used as genotoxic agent and *Nyctanthes arbor tristis* as ameliorating agent.

2.2 Slide Preparation
After the completion of treatment animal were sacrificed by cervical dislocation and slide were prepared from bone marrow cells of the mice by colchicine-hypotonic-acetoalcohol –flame drying-giemsa staining technique [28].

2.3 Slide Screening
Mitotic metaphase chromosome was screened under the microscope for the study of structural and mitosis disruptive abnormalities in each variants. 300 well spread plates of metaphase was screened randomly and student's t- test was applied to evaluate the significant difference among the groups and to find out the protective effect.

Table 1: summary of the treatment protocol

S. No.	Experimental variants	Symbol	Dose
1	Control	C	No dose.
2	Profenofos	P	2mg/ml
3	Nyctanthes arbor tristis leaf extract (25%concentration)	N1	10ml/kgb.w.
4	Nyctanthes arbor tristis leaf extract (75%concentration)	N2	As 3
5	Profenofos + 25% concentration of leaf extract (Lower dose)	P + N1	As 2 and 3
6	Profenofos + 75% concentration of leaf extract (Higher dose)	P + N2	As 2 and 3

3. Results
Both structural and mitosis disruptive types of abnormalities were found in all the variants. Profenofos significantly increased the frequency of chromosomal abnormalities (24%) compared with control (5.3%). When *Nyctanthes arbor tristis* leaf extract (both concentration) administered alone, the abnormalities (6% and 8%) were found almost to the control level. However, when administered lower doses of leaf extract with profenofos concurrently, significantly minimized the abnormalities about half (14.6%) of the profenofos induced genotoxicity, but when higher dose of leaf extract with profenofos significantly minimized (8.6%) at almost control level (table -2). Leaf extract protected both (structural and mitosis disruptive) types of damaged but higher dose of leaf extract was found to be more effective. Hence, this result found to be dose dependent.

Table 2: incidence of chromosomal abnormalities in bone marrow cells of mice treated with profenofos and two different concentration of leaf extract of *Nyctanthes arbortristis.*

Experimental variants	Structural abnormality			Mitosis disruptive abnormality			Total abnormality		
	No.	%	± S. E.	No.	%	± S. E.	No.	%	± S. E.
C	6	2	± 0.08	10	3.3	± 1.02	16	5.3	± 1.29
P	33	11	± 1.80^{abc}	39	13	± 1.94^{abc}	72	24	± 2.59^{abc}
N1	8	2.6	± 0.91	10	3.3	± 1.02	18	6	± 1.37
N2	10	3.3	± 1.02	14	4.6	± 1.20	24	8	± 1.56
P+N1	20	6.6	± 1.43	24	8	± 1.56^{a}	44	14.6	± 2.03^{ac}
P+N2	11	3.6	± 1.07^{b}	15	5	± 1.25^{b}	26	8.6	± 1.61^{b}

a, b, c indicates significant difference with corresponding value in the control, profenofos and *Nyctanthes arbortristis* variants, respectively.

4. Discussion
The results of the studies described above suggest that the profenofos increased the frequency of abnormalities and the concurrent administration of leaf extract at higher and lower dose with profenofos appreciably reduce the genotoxic effect of profenofos. The exact molecular mechanism of genotoxic effect of profenofos pesticide is not known. It is however, believed that the electrophilic centres generated during the metabolic transformation of pesticides may interact with chromosomes by attacking nucleophilic sites on its DNA [29]. Similar finding were reported by El- khatib and shalaby [30] on effect of two pesticides: Alpha-cypermethrin (synthetic pyrethroids) and diazinon (organophosphorus) on rat bonemarrow cells and on white rat [31]. The specific literature regarding the anti-genotoxic effect of Nyctanthes leaf extract is also not known. It is possible that Nyctanthes leaf extract is reported to have antigenotoxic effect due to excellent antioxidant activity. A similar conclusion has been drawn for antimutagenic effect of vitamin C [32], vitamin A [33]. It has been confirmed that plant flavonoids inhibit the mutagenicity induced by chemical mutagen [34]. Antigenotoxic activity has also been reduced due to tannin, these are also phenolic compounds and are widely distributed in plants [35]. The leaf extract of Nyctanthes is source of antioxidants as flavonoids and tanins.

On the basis of this result, it is suggested that leaf of Nyctanthes arbor tristis combat the menace of pesticide induced genotoxicity.

Acknowledgment
The authors are thankful to Professor M.C. Verma, Head, University Department of Zoology, T.M. Bhagalpur University, Bhagalpur for providing laboratory facility on animal system.

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